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CLONED EUKARYOTIC DNA

Introduction

We envisage three ways that recombinant eukaryotic DNA molecules could be hazardous.

1. A gene could function in the bacteria in which it is cloned and produce a toxic product.
2. A DNA component could in someway enhance the virulence or change the ecological range of the bacterium, in which it is cloned.
3. A DNA component could infect some plant or animal, integrate into its genome, or replicate, or by its expression could produce a modification of the cells of the organism.

The vector with which the DNA is recombined, the source of the eukaryotic DNA, and the kind of experiment used to produce recombinant molecules are important variables which we have considered. Mainly we discuss potential hazards of laboratory experiments, and these may increase when recombinant DNAs of known function are used for some applied medical, or agricultural purpose. We have commented on this at the end.

Our assessment of the biohazards of recombinant DNAs has been made in terms of the class of protection which might be required for any particular experiment, using the different levels defined by the plasmid group.

1. Vectors.

The great majority if not all of the in vitro recombinant DNA molecules that have been constructed to date have been propagated in E. coli and have, perforce, required plasmids or bacteriophage as their vectors. We have assessed potential biohazards for various types of donor fragments used with these systems, which we would regard as exemplifying prokaryotic vector and host systems. These are illustrated in Table 1.

Many of the future developments in the cloning, amplification, and utilization of eukaryotic DNA sequences will, however, require other vectors and host cells. The long term objectives of gene therapy, for example, can only be achieved by transfer of new sequences to animal cells, for which the obvious vectors are animal viruses. Similar systems are attractive for furthering the basic molecular biology of eukaryotic cells, both animal and plant, but they immediately present an additional range of potential biohazards.

Clearly the discovery or even construction of a DNA vector for simple eukaryotes would be of very great advantage for this work.

Will the hazards attending the use of eukaryotic vectors and host cells be greater or less than those arising in experiments where the same DNA fragments are manipulated in prokaryotic systems? Only time and experience can provide the necessary information on this, but since the transfer of new sequences to animal host cells via animal viruses places the new sequences directly in the type of target area that we wish to avoid when using prokaryotic systems to amplify selected DNA fragments, we can only assume that a potential biohazard inherent in a given DNA fragment is likely to be greater when propagated in the animal system. This is reflected in the higher rating which is in general assigned in Table 1 to a given type of DNA fragment in such an instance. Normally, the conditions under which such experiments might be conducted would at least match those used for work with the animal virus concerned.

Plant virus and host cells present some difficulty and we have attempted to make a conservative assessment in the light of common handling practice for plant viruses. Hence, the consensus was that the systems are less likely to present the degree of biohazard potential encountered with animal cell systems, but that it would be prudent to equate them with at least the level accorded to corresponding experiments with prokaryotic vectors and host cells.

2. Eukaryotic DNA molecules

In general the level of precautions employed in experiments involving incorporation of eukaryotic virus genomes should not be less than that appropriate for the donor virus itself.

"Shotgun" experiments involve the production of recombinants between a vector and total eukaryotic DNA. This kind of experimentation will be used extensively to isolate a wide variety of eukaryotic DNA components. Mammalian DNAs, primates in particular, were rated high in biohazard because DNAs from these sources are more likely to contain infectious agents pathogenic for humans. Shotgun experiments involve DNA of unknown function, and introduce into the bacterium new sequences with unpredictable consequences. These considerations place all shotgun experiments in Class 3 or higher regardless of the source of DNA.

Purified DNA components are distinguished in biohazard severity by whether or not they are expressed in the bacterium. Some class assignments may seem high but this conservative assignment reflects our ignorance of how eukaryotic DNAs will function within bacteria. We suggest that a eukaryotic gene for a protein usually will not be transcribed nor translated faithfully inside bacteria without additional genetic manipulations. We have

also listed the kinds of conditions and information which would lead to a reclassification of the recombinant DNA. Finally, we describe some contract experiments which should be performed to inform us about the hazard potential of these DNAs.

The table shows the consensus of the committee on the class of protection required for different types of experiments. Concordance was good. The levels of protection are those defined by the plasmid group. It was recognized that the definition of class 3 protection is wide, leaving much to the discretion of the investigator. Some members assigned certain experiments to class 6, but we later agreed that this would be hard to implement; nevertheless we all felt that there were some experiments, such as joining a high risk virus to an animal vector, which would require very strong scientific justification to be done at all.

Purified DNA derived from any of these experiments has not been considered separately. In view of our ignorance of the infectivity of cloned recombinant DNA molecules it would be prudent to destroy such preparations before disposal.

Reassessment of Hazard Class Assignments

The initial classification of experiments will be made largely in ignorance of actual hazards and should be reevaluated in the light of subsequent experience. Some examples of instances in which these classifications could be reassessed are:

A. Possible reassignment to a lower class.

1. "Safe" bacterial carriers or plasmid vectors are used; examples are given in the plasmid group's report.
2. It is demonstrated that the eukaryotic DNA segment is not expressed.
3. Individual recombinant DNAs from a shotgun experiment are shown to contain only genes of known function and these are expected to be minimally hazardous.
4. Tests demonstrate that anticipated biohazards have not materialized (e. g. , recombinant plasmid DNA containing human DNA fragments is shown to be incapable of transforming or infecting human cells). Other examples are given in the plasmid group's report.
5. The portion of an animal virus DNA in a particular recombinant plasmid contains only genes which are expected to present lesser hazards than the whole viral genome.

B. Possible reassignment to a higher class.

1. For special purposes, more pathogenic bacteria or less desirable vectors (e. g. , F factors) are used.
2. It is discovered that the ecological potential or virulence of the bacterial carrier has been increased by the presence of the recombinant plasmid.

3. Alterations of the plasmid or the carrier are initiated or accomplished which would increase the probability of expression of the eukaryotic DNA.

4. A recombinant plasmid derived from a shotgun experiment is found to contain sequences homologous to eukaryotic virus genomes.

5. A strain considered to be relatively safe is mutagenized.

Hazards Associated with Large Scale Applications

Although it may be that eukaryotic genes are not normally transcribed and/or translated with fidelity in bacteria we presume that such conditions can be obtained by genetic manipulation of the bacterial genome, or of the vector-eukaryote hybrid DNA. Numerous applications of this kind of eukaryotic gene activity have been suggested - for example, the bacterial production of insulin in pharmaceutical factories. These applications will generally involve the growth of very large numbers of the relevant bacteria, and the eukaryotic gene products they contain may well be hazardous to the general population.

The problems of containment associated with these applications are likely to be increased substantially over those considered previously. We therefore set them apart from the hazard ratings given above. We recommend that such applications be undertaken only after it can be demonstrated that the bacteria are "safe"; that is, they will not be hazardous even if they escape the confines of their intended use. The concept of safe bacteria is discussed in the report by the plasmid group.

APPENDIX

Some Experiments Relevant to Hazard Assessment

1. Will a eukaryotic gene function properly in a bacterium?

From the available data, it seems unlikely that E. coli RNA polymerase will generally recognize eukaryotic promotor, initiation or termination sequences in DNA, nor do E. coli translation factors generally recognize starting signals in eukaryotic mRNAs. This is certain to be an area of intense investigation.

2. Can DNA and RNA infect animals or plants? This could best be answered with viral nucleic acids since a single infective event is greatly amplified and readily assayed. All means of entry of the nucleic acid should be tested. The survival of the biological potency of DNA may be assayed in higher organisms by using transformable bacteria growing in the animal or plant.

3. Can hybrid DNA molecules or their transcripts be transmitted from bacterial cells to animals or plants? Again probably the most sensitive probe would be a hybrid between a plasmid and a plant or animal viral genome. Knowledge of the answer to question 2 is essential since transmission could occur by the release of DNA or RNA from bacterial cells. All forms of transmission from the bacteria containing the hybrid molecule should be tested.

4. Can a hybrid DNA molecule in a phage particle infect an animal or plant cell? A phage particle, being a highly condensed and protected form of the DNA, may be more readily taken up by eukaryotic cells. Again probably the most sensitive probe would be a hybrid between bacteriophage λ and a plant and animal viral genome.

5. Can "safe" vectors or cloning cells be constructed? A "safe" vector or cloning cell may be designed to self destruct upon "escaping" from a laboratory. For example a number of conditional lethal mutations in essential functions may be incorporated into the cloning cell and vector. Also one might incorporate a restriction endonuclease gene without the methylase gene into the vector. Such a vector could only then propagate in the corresponding methylating cell.

6. Can human cells in culture be transformed by human DNA fragments, or any other DNA?

7. Will bacteria containing eukaryotic DNA recombinant molecules, have altered ability to colonize any eukaryote?

8. How frequently can the plasmids or phage used in cloning experiments be transfered to other bacterial cells?

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TABLE I

CLASS ASSESSMENT FOR DIFFERENT KINDS OF EXPERIMENTS

DNA	VECTOR		
	EUKARYOTIC		PROKARYOTIC
	ANIMAL	PLANT	
1. Shotgun experiments - DNA or transcripts of RNA			
DNA: Primate	5	4	4
Mammalian	4	4	4
Other vertebrate	4	3	3
Invertebrate	4	3	3
Higher plant	4	3	3
Simple eukaryote	4	3	3
2. Purified DNA or transcripts of purified RNA from viruses[1]			
High risk animal viruses	5	5	5
Moderate to low risk animal viruses	4	4	4
Plant viruses	4	3	3 or 4
3. Purified eukaryotic sequences - DNA or transcripts of RNA			
(a) Expressed in host of vector[2]			
1. Known toxic products	5	5	5
2. Other products	4	3 or 4	3
(b) Not expressed	4	3	2

[1] Use of partial genomes might lead to reevaluation.

[2] The experiments would always have to be done on the initial assumption of expression.